Controlling Scaffold Conductivity and Architecture to Direct Myogenic Cell Alignment and Differentiation

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Statement of Purpose: Skeletal muscle's combination of three-dimensional (3D) anisotropy and electrical excitability is critical for enabling normal movement. However, both the structural organization and electrical properties of muscle are disrupted following injury. We previously developed a highly aligned 3D collagen scaffold incorporating conductive polypyrrole (PPy) particles to recapitulate these key muscle properties and showed that the scaffold facilitated enhanced myotube maturation compared to non-conductive controls[1]. To further optimize this scaffold design, this work aimed to assess the influence of conductive polymer incorporation and scaffold pore architecture on myogenic cell behavior.

Methods: Aligned collagen-glycosaminoglycan scaffolds, with or without conductive polymers PPy or poly(3,4-ethylenedioxythiophene) (PEDOT), were fabricated by directional lyophilization. Scaffolds with varying pore microstructure were created by modulating freezing temperature during lyophilization. Scaffold conductivity was analyzed via linear sweep voltammetry (LSV) and conductive polymer localization was evaluated using energy dispersive spectroscopy (EDS) and SEM. Pore diameter was quantified using MATLAB. C2C12 mouse myoblasts and human muscle progenitor cells (MPCs) were cultured within scaffolds with metabolic activity quantified using AlamarBlue. Cell organization and differentiation were determined by confocal imaging of F-actin and myosin heavy chain respectively.

Results: Combined EDS and SEM analyses confirmed the homogeneous presence of PPy and PEDOT in conductive scaffolds by increased chlorine (Cl, from the iron chloride dopant in PPy) and sulfur (S) peaks respectively (*Fig. 1A*). The addition of 0.5 wt% PPy or 1 wt% PEDOT resulted in conductivities of 1.83 ± 0.40 and 1.82 ± 0.21 mS/m respectively while non-conductive CG scaffolds possessed a conductivity of 0.59 ± 0.07 mS/m (*Fig. 1B*). Myoblast culture over one week within PEDOT-doped scaffolds showed superior metabolic activity compared to PPy scaffolds that was more than double day 0 levels by day 7 (*Fig. 1C*). However, metabolic activity was still reduced compared to non-conductive scaffolds. Confocal imaging showed alignment and cytoskeletal extension of human MPCs along the scaffold backbone (*Fig. 1D*). Additionally, motivated by previous work highlighting the influence of scaffold pore size on cell proliferation and gene expression[2], we tuned scaffold pore diameter by control of the freezing temperature during lyophilization. This produced scaffolds with increasing pore size as freezing temperature was increased (116.6 ± 16.8 µm, -50ºC; 139.8 ± 14.0 µm, -30ºC; 195.1 ± 20.0 µm, -20ºC; 234.2 ± 23.3 µm, -10ºC; *Fig 1E*). Aligned scaffold organization was maintained independent of freezing temperature.

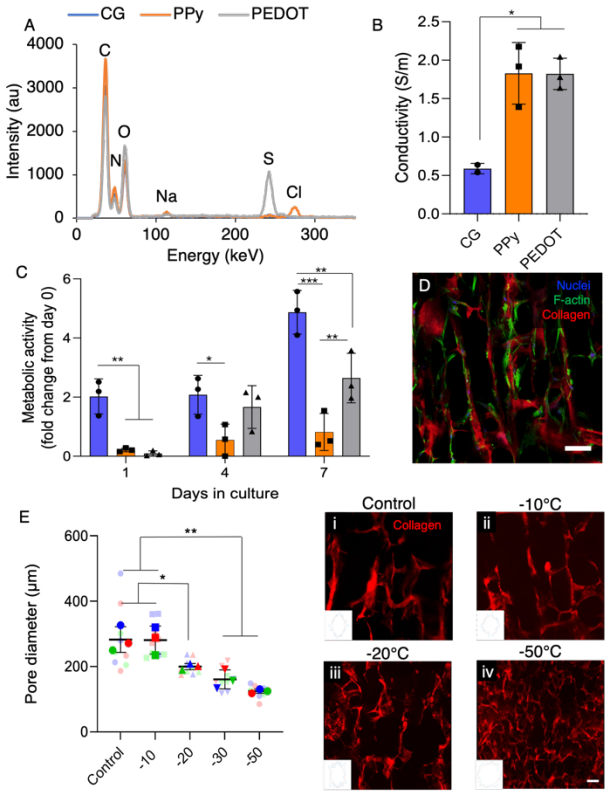

Figure 1. A) EDS spectra of conductive scaffolds shows S and Cl peaks indicative of PEDOT and PPy incorporation respectively. B) LSV data shows increasing scaffold conductivity with addition of 0.5 wt% PPy or 1 wt% PEDOT. C) PEDOT scaffolds support superior cell metabolic activity compared to conductivity-matched PPy scaffolds, although both groups show lower metabolic activity than non-conductive controls. D) Human MPCs conform to the aligned scaffold contact guidance cues. E) Decreasing lyophilization temperature results in conductive scaffolds with smaller pore diameter. i-iv: representative images of scaffold pore structure. Scale bars: 100 µm. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

Conclusions: In this study we assessed how conductive polymer incorporation and pore microstructure affected myogenic cell metabolic activity and organization. The inclusion of both PPy and PEDOT led to superior material conductivity, however only PEDOT supported sustained and increasing myoblast metabolic activity. Modulating freeze drying parameters enabled production of scaffolds with a range of different pore sizes. Ongoing work aims to optimize human MPC differentiation and myotube maturation within the conductive scaffolds.

References: 1) IM Basurto et al., *Biomater. Sci.*, 18, 2021; 2) SR Caliari et al., *Biomaterials*, *32*, 2011.